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Potential evidence of fossilised Neoproterozoic deep life: SEM observations on calcite veins from Oppaminda Creek, Arkaroola, South Australia

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Abstract Scanning electron microscopy revealed micron-sized globular and coccoid objects, associated with filaments and mucus-like patches in antitaxial fibrous calcite veins from Oppaminda Creek, Northern Flinders Ranges, South Australia. Chemically the objects only differ from their calcite ($CaCO_3$) matrix by a higher sulphur content. The ~585 Ma veins formed at about 3–6 km below the surface. Fluid inclusions indicate a temperature of formation of about 60–80°C, and not exceeding 100°C. A non-biogenic origin of the objects is discussed, but considered unlikely. Instead, morphology, chemistry and size distribution all indicate that the objects are fossilised microbes that lived in the veins at the time and depth of vein formation.

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Introduction

Compared to other fossil groups, records of fossilized microbes are relatively rare in the literature and in some cases the subject of strong controversy (e.g. Schopf 1993; Brasier et al. 2002; García-Ruiz et al. 2002). The main reason is the generally low preservation potential of microbes during diagenesis and lithification of sediments. Up to now, the search for fossil microbes mainly focused on rocks and minerals from surface and near-surface environments, such as stromatolites and cherts (Schopf 1993; Brocks et al. 1999; Rasmussen 2000; Kazmierczak and Altermann 2002; Van Kranendonk 2006; etc.). Although microbes are known to inhabit subsurface sediments and rocks, even down to several kilometres depth (Pedersen 1993; Stetter et al. 1993; Stevens and McKinley 1995; Liu et al. 1997; Kerr 2002; Stetter 2002; Lin et al. 2006), this environment has been relatively neglected so far in the search for fossil microbes. A problem is to define prospective *Lagerstätten* for microbes that once lived well below the surface. Even if suspected fossil "deep life" microbes are found in rocks, it is difficult to ascertain when they lived and whether they indeed lived well below the surface or that they lived in surface sediments that were subsequently buried.

Determining the age of fossil deep life is virtually impossible, unless the age of the hosting material can be dated and proven to have formed well below the surface. Mineral veins form a potentially promising host for preserving traces of deep life, because the conditions of their formation and often their age can be determined by various

techniques (fluid inclusions, isotope geochemistry, etc.). Furthermore, the minerals in veins may encapsulate contemporaneous microbes and ensure their preservation. In this paper we report on suspected fossil microbes that were found inside calcite crystals in late Neoproterozoic antitaxial veins from South Australia. For readers that are less acquainted with the different vein types and in particular the uncommon *antitaxial* one that is subject of this paper, we provide a short overview on veins, before describing the studied veins and the suspected fossil microbes.

Mineral veins

Mineral veins are common structures in rocks. They are usually planar structures filled with one or more minerals (typically calcite or quartz), which precipitated from a fluid (see e.g. Jamtveit and Yardley 1997; Bons 2000; Oliver and Bons 2001 for recent reviews on the formation of veins). Most veins formed by mineral precipitation inside open fractures in rocks. These fractures both served as conduits for the mineralising fluids and space for minerals to precipitate in (e.g. Etheridge et al. 1984; Oliver 1996; Cox et al. 1986, 2001). Precipitation of minerals may be driven by changes in fluid pressure or temperature, chemical composition of the fluid (mixing) or host rock (e.g. Boullier et al. 1994; Giles et al. 2000). Compaction of a rock can lead to dissolution of minerals at grain contacts and diffusional transport to open fluid-filled fractures, where the minerals precipitate again (e.g. Gratier 1987; Fisher and Brantley 1992; Renard et al. 2000). Finally, microbial activity can also drive mineral precipitation inside rocks (e.g. Hofmann and Farmer 2000; Budai et al. 2002).

Vein classification

Based on the internal vein structure, Durney and Ramsay (1973) defined three main types of veins: *syntaxial* veins, *stretched-crystal* veins (named "*ataxial*" by Passchier and Trouw 1996), and *antitaxial* veins. Crystals within syntaxial veins grow on the surface of a fracture towards the centre of the vein (Fig. 1a). Crystallographic growth competition produces a continuous increase of the average crystal width towards the centre of the vein. Ataxial or stretched-crystal veins form by the repeated fracturing and sealing of a rock, the so-called "crack-seal mechanism" of Ramsay (1980). Each crack event forms a narrow fracture, which is subsequently sealed by growth of the minerals that line the fracture (Fig. 1b). The result is a vein with long columnar crystals, which are "stretched" by the many crack-seal events. The crystals typically have serrated boundaries and show no consistent growth direction. Because the vein-forming mineral grains precipitate onto existing wall rock grains, syntaxial and ataxial veins tend to have similar minerals as the wall rock, like quartz veins in a quartzite or calcite veins in a limestone.

Antitaxial fibrous veins

Antitaxial veins (Fig. 1c) form the third and most uncommon and enigmatic vein type. Widening of an antitaxial vein takes place by outward growth of existing vein crystals at the two outer surfaces of the vein (Durney and Ramsay 1973). This means that the oldest vein precipitate is found in the centre of the vein, in the median zone, and the precipitate gets progressively younger towards the outer surfaces of the vein. Another typical characteristic of antitaxial veins is the almost complete lack of growth competition, which leads to a strongly fibrous habit of the crystals, even in minerals that are not normally fibrous, such as calcite (Bons and Jessell 1997; Bons 2000; Hilgers et al. 2001). The fibres have smooth boundaries, contrary to

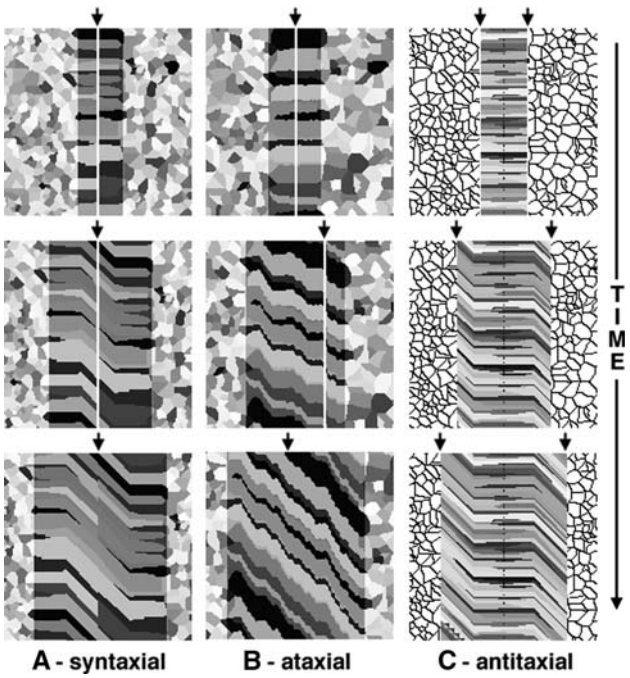

Fig. 1 Three main vein types, each at three stages of their development. *Arrows* indicate the plane(s) where material is added to the vein. **a** Syntaxial vein growth with precipitation in a fracture usually located somewhere in the middle of the vein. **b** Ataxial vein formation with repeated fracturing and sealing at different sites within the growing vein. **c** Antitaxial growth with crystals growing outwards against the wall rock, which normally is of a different mineral composition than the vein minerals. In all three cases, wall rock divergence started at a right angle to the vein and then rotated to oblique divergence

fibres in stretching veins. Because growth occurs in both directions from seed crystals in the median zone, fibres can span the whole width of the vein. This never happens in syntaxial veins, because crystals grow towards the centre of the vein, where they abut against other crystals growing from the opposite direction. Finally, contrary to syntaxial and ataxial veins, antitaxial veins tend to have a mineral composition that distinctly differs from their host rock, like calcite veins in a shale.

Until recently it was commonly assumed that antitaxial fibrous veins formed by the crack-seal mechanism of Ramsay (1980). However, consensus is now building that antitaxial veins grow in the absence of open fractures at the outer surfaces where growth takes place (Bons and Jessell 1997; Bons 2000; Means and Li 2001; Wiltschko and Morse 2001; Elburg et al. 2002; Bons and Montenari 2005; Hilgers and Urai 2005; but also see Mügge 1925, 1928). The process of antitaxial growth requires a substrate in the centre of the vein on which overgrowth can take place. Antitaxial veins therefore always have a narrow median line or zone, which has a different texture and forms the seed for subsequent antitaxial growth (Durney and Ramsay 1973). The different texture indicates that median zones usually form as thin syntaxial or ataxial veins in a fracture (Bons and Montenari 2005). Growth on a closed, mechanically coherent surface by mineral precipitation from a supersaturated pore fluid is possible by the "force of crystallisation" (Taber 1916; Fletcher and Merino 2001; Hilgers and Urai 2005). Wiltschko and Morse (2001) showed that geologically realistic supersaturations can easily provide large enough forces to push apart the wall rock.

Although antitaxial veins are not common, they have been found in many localities around the world, for example in Mesozoic and Tertiary shales in the Alpine nappes in Switzerland (Ramsay et al. 1982; Ramsay and Huber 1983), Palaeozoic shales in the Appalachians of New York State (Passchier and Urai 1988; Urai et al. 1991), Cretaceous shales at Sestri Levante, Italy (Marroni 1991), in the Burgess Shale, Canada, and in Neoproterozoic shales in the Northern Flinders Ranges, South Australia, which are subject of this paper. All these occurrences are within dark shales, rich in organic matter, carbonate and sulphides (usually pyrite). The main vein-forming mineral is calcite in all cases, with minor quartz and phyllsosilicates. The latter minerals can grow side-by-side with the calcite fibres, but are usually constrained to the outer rim of the veins, where they grow syntaxially from the wall rock into the vein (Hilgers and Urai 2002).

To gain insight into the genesis of antitaxial veins, we carried out a detailed study of an occurrence of abundant antitaxial calcite veins at Oppaminda Creek in the Northern Flinders Ranges. Structural–geological (Bons and Montenari 2005) and geochemical analyses (Elburg et al. 2002) so far showed that these veins grew at a depth of 3–6 km below the sediment surface at about 585 Ma. The veins derived their carbonate partly from the local wall rock and partly from external fluids that percolated through the rock (Elburg et al. 2002). Here we present new fluid inclusion data, chemical analyses and in particular scanning electron microscopy results. Based on these data, we argue that the veins were probably inhabited by microbes at the time of their formation.

Description of the veins

Regional setting

Antitaxial calcite veins are abundant in the Tindelpina Shale Member of the Neoproterozoic Tapley Hill Formation, which outcrops well at Oppaminda Creek, a few km southeast of Arkaroola Village in the Northern Flinders Ranges (South Australia) (Fig. 2). The Tapley Hill Formation forms part of the Neoproterozoic to Cambrian Adelaidean Sequence (Coats and Blissett 1971; Preiss 1987; Drexel et al. 1993), and was deposited at about 643 ± 2.4 Ma (Kendall et al. 2006), following the Sturtian glaciation (Preiss 1987). The shales are dark, very finely laminated (Fig. 3a), and rich in carbonate, organic matter and pyrite (McKirdy et al. 1975). Sedimentation continued in the "Adelaide Geosyncline" basin until the Cambrian. The whole Adelaidean Sequence was subsequently involved in the ~500 Ma Delamerian orogeny (Drexel and Preiss 1995). Delamerian deformation and metamorphism is very variable. In some parts of the fold belt, especially in the south (Kangaroo Island, Adelaide Hills), deformation was intense and metamorphism reached amphibolite facies (Coats and Blissett 1971; Preiss 1987; Drexel and Preiss 1995). In the area around Oppaminda Creek, however, Delamerian deformation was only minor (Paul et al. 1999). Here, kilometre-scale open folds produced minor tilting of layers in the absence of any cleavage development or metamorphic overprint. The metamorphic peak in the study area did not exceed ~200°C. Metamorphic grade and deformation intensity slowly increases from the study area towards the Proterozoic Mt. Painter Inlier to the north, where amphibolite-grade is reached (Mildren and Sandiford 1995; McLaren et al. 2002). A Late Ordovician magmatic-thermal event (Elburg et al. 2003; Bakker and Elburg 2006) affected the general region, particularly to the north, but produced no noticeable metamorphic overprinting of the shales at Oppaminda Creek.

The 350 m thick Tindelpina Shale Member that carries the calcite veins is well exposed in Oppaminda Creek and other dry creeks that cut through the gently (~20°) west-

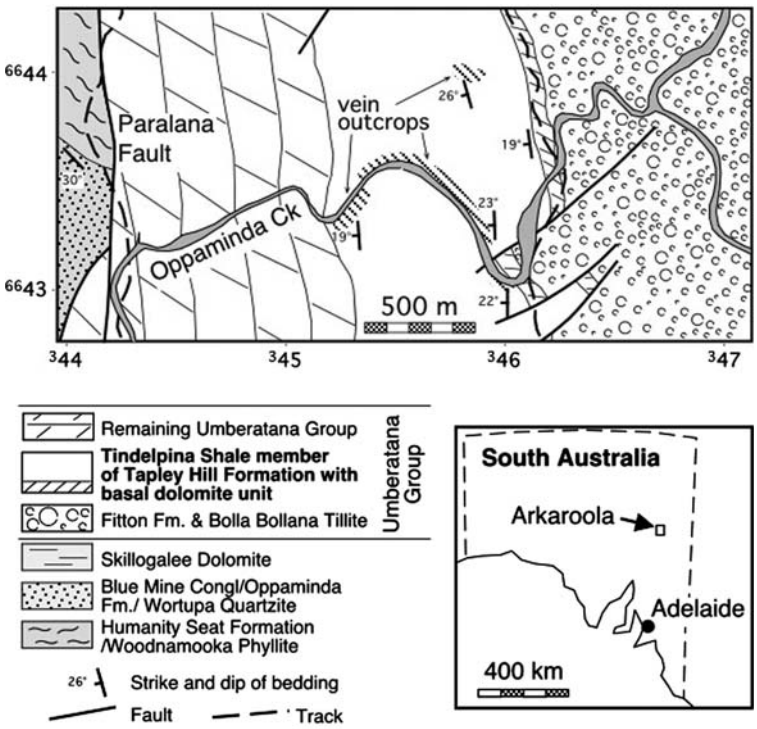

Fig. 2 Geological map of the sampling area SE of Arkaroola Village in the Northern Flinders Ranges, South Australia. Veins occur in the W-dipping Tindelpina Shale Member at the base of the Tapley Hill Formation, part of the Neoproterozoic Umberatana Group. Based on Preiss (1987) and own field work

dipping unit. Vein samples were collected from Oppaminda Creek and an unnamed small creek a few hundred metres further north (Fig. 2).

Macroscopic and microscopic description of the veins

Veins are usually a few mm to a few cm thick, reaching a maximum width of 10 cm (Fig. 3). Their length can vary from a few centimetres to many metres. The veins occur in parallel and branching sets, with a large variation in orientation (see Bons and Montenari (2005) for a detailed structural–geological analysis).

The dominant vein type is antitaxial and fibrous (Fig. 3c). The veins all have a calcite-filled median zone (usually a few to tens of micrometres, occasionally reaching up to about a mm), relatively rich in wall–rock inclusions and with a non-fibrous internal structure. Calcite fibres grew outward in both directions from these median zones (Fig. 3d, e). Calcite fibres usually have widths in the order of tens to hundreds of μm, rarely more. There is a minor increase of fibre width from the centre to the rim of the vein, indicating that the growth direction was outwards from the median zone. The fibre boundaries are smooth. The fibres tend to be smoothly curved, as is normal for antitaxial fibrous veins (Durney and Ramsay 1973). The curved fibres track the opening trajectories of the veins. Curvature is not the result of later deformation, because the crystallographic lattice is not bent (Urai et al. 1991). Very minor deformation of the veins is indicated by deformation twins in the calcite.

The veins consist of almost pure calcite, with minor (<1% each) Fe- and Mg-carbonate (Elburg et al. 2002). Pyrite, chalcopyrite and Fe-(hydr-)oxides are present in trace amounts, with rare macroscopically visible crystals. The fibrous veins all have a thin rim of quartz crystals, which grew inwards from the wall rock (Fig. 3c). The fibre boundaries usually end at the tips of quartz crystals on the rim of the veins. This was previously explained by these boundaries locking onto these asperities during their growth (Urai et al. 1991; Hilgers and Urai 2002; Bons and Montenari 2005). However, detailed optical analysis indicates that the quartz crystals are younger and overgrow the calcite fibres. This is confirmed by fluid-inclusion analyses (see below). Calcite- and dolomite-bearing veins with a non-fibrous, blocky texture also occur in the same shales, but overgrowth relationships show that these post-date the antitaxial fibrous veins.

Age and depth of vein formation

Elburg et al. (2002) presented ten ages, ranging from 497 to 622 Ma (average 565 Ma) for which the Sr fraction from the leached host rock of veins would be in equilibrium with the vein Sr. The range of ages overlaps with a 586 ± 30 Ma basin-wide fluid flow event that was reported by Foden et al. (2001) for the region. The age of the veins is therefore

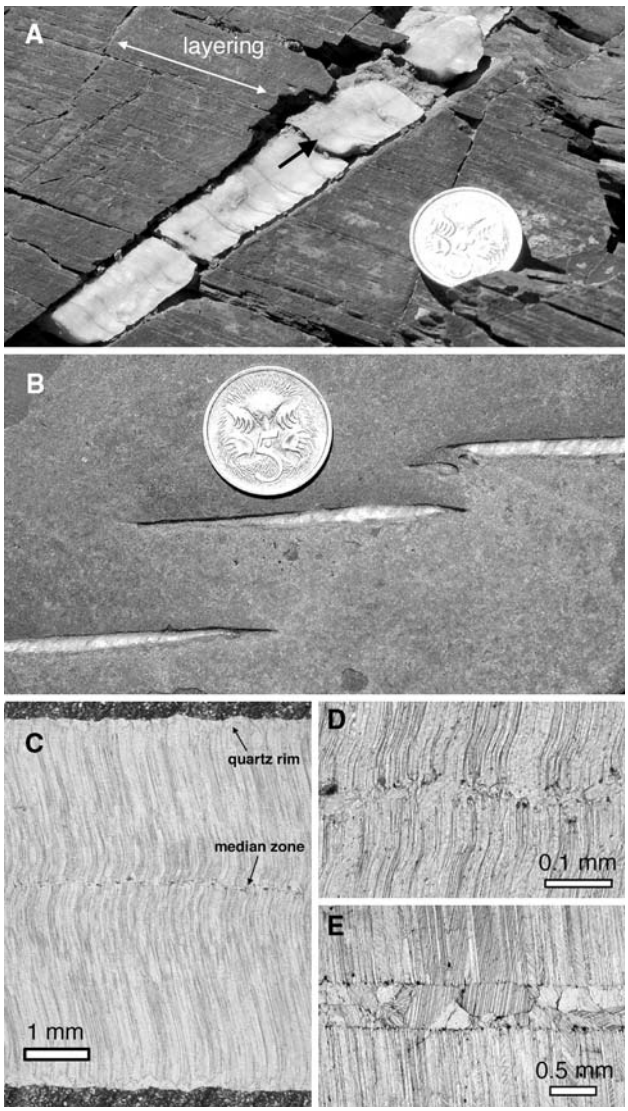

Fig. 3 **a** Straight fibrous vein with a thin median zone (*black arrow*). Host shale is clearly thinly laminated and undeformed, apart from being tilted. **b** Lenticular fibrous veins, looking down on bedding surface of shales. Ø 5 cent coin is 19 mm. **c** Photomicrograph of antitaxial fibrous calcite vein. Curved fibres grow out from the median zone, recognisable by small wall rock inclusions. **d** Close up of narrow median zone of vein shown in (**c**). **e** Detail of a wider median zone showing that its internal structure is distinctly different from the younger fibrous calcite on either side

estimated by Elburg et al. (2002) to be about 585 Ma, at which time the Upper Adelaidean Wilpena Group sediments were being deposited in a shallow coastal environment (Drexel et al. 1993), approximately at the start of the Ediacaran.

Fibres in antitaxial veins generally track the "opening trajectory" of the veins (Durney and Ramsay 1973). This means that the fibre orientations show the direction in which the walls of the veins diverged, which relates to the direction of tectonic extension experienced by the host rock at the time of vein formation. Bons and Montenari (2005) showed that the extension direction gradually moved towards a NNW–SSE extension, incompatible with the local Delamerian shortening in the same direction (Paul et al. 1999). A pre-Delamerian age of the veins is therefore also supported by the structural data.

The height of the sediment column above the Tindelpina Shale Member at about 585 Ma was estimated at 4–6 km (uncorrected for compaction) by Bons and Montenari (2005), using isopach maps of Preiss (1987) and a profile constructed from the geological map of Coats and Blissett (1971). However, these sources probably overestimate the thickness of the sediment pile by up to ~1 km, according to W.V. Preiss (personal communication, 2004). The estimated depth of vein formation is therefore constrained to about 3–6 km below the seafloor.

Methods

Fluid inclusions

Doubly polished thick sections were prepared from the fibrous calcite veins. A Jobin Yvon LABRAM confocal-Raman spectrometer equipped with a frequency-doubled Nd-YAG laser (100 mW, 532.2 nm) with an LMPlanFI 100×/0.80 objective lens (Olympus) was used to identify fluid and solid phases in inclusions. Microthermometric measurements were carried out with a Linkam MDS 600 stage operating over a temperature range from –100 to + 250°C. Synthetic fluid inclusions were used for the calibration at –56.6, 0.0 and 374.0°C. Volume fractions were obtained from area analysis in a two dimensional projection of fluid inclusions (Bakker and Diamond 2005).

Scanning electron microscopy

Blocks of fibrous veins and their wall rock were examined with a scanning electron microscope (SEM). The polished blocks were etched with 0.1 molar HCl for 30 s to remove the outer ≤25 μm calcite, thus removing any recent contamination and revealing inclusions that are fully embedded within the calcite crystals (Fig. 5a). This method has formerly been used by e.g. Chafetz and Folk (1984) to reveal bacteria enclosed in travertine calcite. Etched samples were immediately rinsed with filtered distilled water and oven-dried. After drying, the samples were sputter-coated with gold for 30 s (Figs. 5, 6), except one un-coated sample that was analysed with a field emission SEM that does not require the conductive gold layer (Fig. 8). In order to reveal the morphology of the calcite fibres, one sample was not etched. Gold-coated samples were analysed with a

LEO-Gemini 1450VP SEM (Tübingen University), at voltages between 15 and 30 kV. Energy dispersive spectroscopy (EDS) was carried out at 15 kV and a working distance of 15 mm. Uncoated samples were analysed with a JEOL JSM-6340F field emission SEM at 2 kV (ExxonMobil, Brussels).

Results

Fluid inclusions

Fluid inclusions within the veins were analysed to determine their trapping temperature and fluid composition (Bakker and Bons 2005). Primary fluid inclusions in the calcite fibres have negative crystal shapes, either flat or three dimensional, and are up to 20 µm long (Fig. 4a–c). The majority of the inclusions have sizes between 5 and 10 µm. About 50% of these inclusions are completely filled with an aqueous liquid, whereas the rest contain small moving bubbles that fill less than 5 vol.% of the total inclusion volume. The total homogenisation (T_H) of these inclusions into the liquid phase occurs mostly between 60 and 80°C (Fig. 4d). Cooling after total homogenisation did not result in nucleation of the vapour bubble again, therefore measurements could not be repeated and only a few inclusions per sample could be measured. At room temperature, the inclusions remain with metastable absence of the vapour bubble. Freezing of the samples usually resulted in decrepitation as ice nucleated between –37 and –42°C. The final ice melting temperature ranges between –2.5 and 0.0°C, indicating a very low salinity to nearly pure H_2O fluid. Gases, like CO_2, CH_4 and H_2, within the fluid inclusions could not be detected with Raman spectroscopy. However, this should not be taken as evidence of absence of these compounds, because the signal from the fluid inclusions was in general obscured by the high fluorescence of the calcite.

Some of the fluid inclusions have been re-equilibrated during a thermal overprint caused by hot fluids, from which quartz, dolomite and calcite precipitated. These phases grow on the fibrous calcite as substrate or replace the fibrous calcite. They are clearly recognisable and identifiable as younger precipitates by their different primary fluid inclusion contents and texture: blocky instead of fibrous. The density of some of the primary inclusions in the fibres was modified due to the temperature increase and reveal T_H values up to 140°C (Fig. 4d), approaching those values observed in fluid inclusions in the younger blocky calcite, which may reach 200°C. Primary inclusions in blocky calcite are completely filled with a hypersaline brine (23–24 equivalent mass% NaCl), similar to the fluid found in quartz and dolomite.

Most of the primary fluid inclusions in the fibrous veins are completely filled with an aqueous fluid. As illustrated with heating experiments, vapour bubbles remain absent after total homogenisation. Therefore, it is suggested that absence of the vapour bubble in many of the primary inclusions represents a metastable fluid state. The homogenisation temperatures between 60 and 80°C reflect the minimum temperature conditions of trapping of all primary fluid inclusions within the fibres. Maximum trapping temperature probably did not exceed 100°C. The temperature range for primary vein formation is in accordance with the

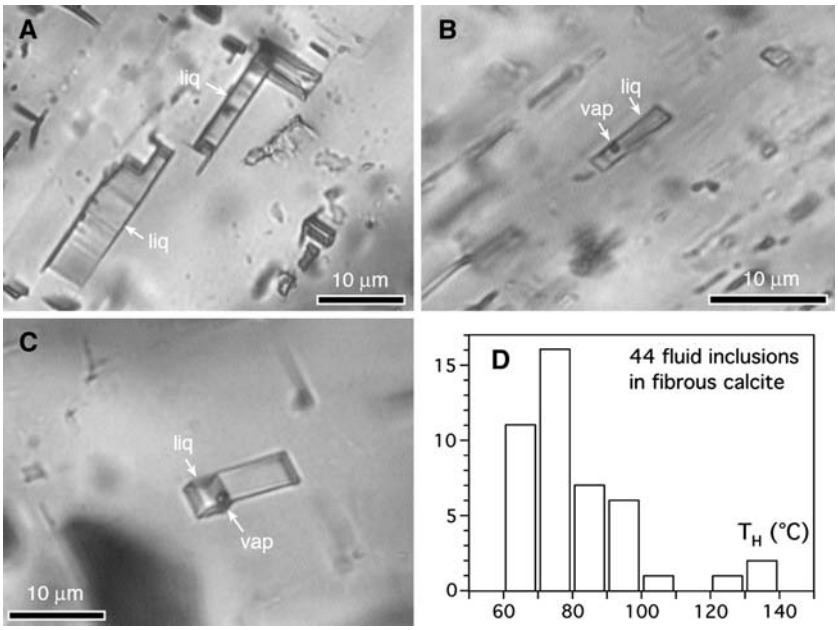

Fig. 4 **a–c** Fluid inclusions in the fibrous vein calcite. The fluid inclusions have negative crystal shapes and some contain a small vapour bubble. **d** Histogram of homogenisation temperatures (T_H) for primary fluid inclusions in the fibrous calcite

3–6 km depth of formation estimate (see above) and a normal geothermal gradient in the order of 20°C/km.

Blocky calcite, quartz and dolomite with high-salinity fluid inclusions and higher homogenisation temperatures, up to 200°C, probably formed later when the rocks were more deeply buried and were affected by the Cambrian Delamerian Orogeny and/or the Late Ordovician thermal-magmatic event (Elburg et al. 2003; Bakker and Elburg 2006).

SEM: secondary electron imaging

SEM images of an un-etched sample show that the fibre boundaries are smooth surfaces and that the fibres are densely packed (Fig. 5b). The fibre boundaries are <<1 µm wide. Etching with HCl removed a layer of calcite and penetrated deeper along the fibre boundaries (Fig. 5a). Only calcite is dissolved by the brief etching, and other materials and minerals are not affected. The result is a rough "micro-landscape" which reveals non-calcite objects that were fully enclosed by the calcite before etching. Objects that are indigenous to the calcite (not introduced after calcite precipitation) can be recognised by the fact that they may still be partly buried inside the calcite.

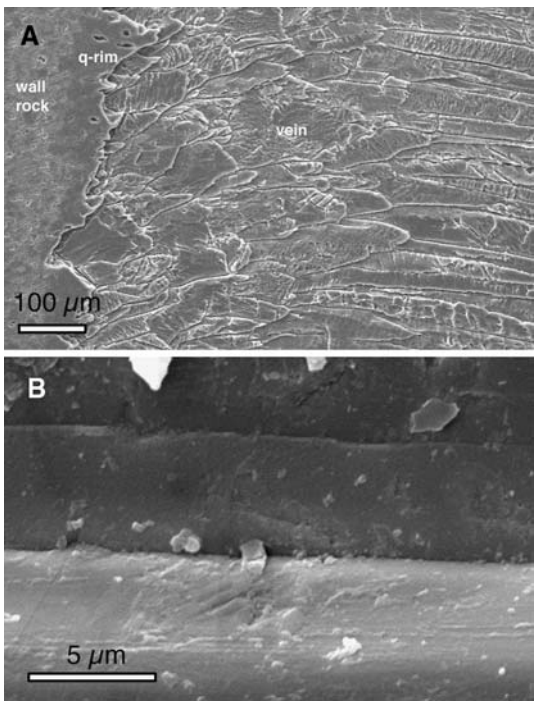

Fig. 5 **a** Overview of vein (*right*)—wall rock contact (*left*) in etched sample. A thin rim of quartz grows into the vein (*dark even grey*). The calcite in the vein has a rough surface due to the etching. Fibres appear short, which is because the fibres are cut obliquely. 30 kV, working distance 20 mm. **b** Close-up of non-etched fibres inside a vein showing the tight packing of the smooth-walled fibres

Apart from mineral inclusions with a clear crystallographically controlled morphology (cubes, needles), the SEM revealed a number of unidentified small structures or objects. Most abundant are ~1 µm-sized globular objects that usually occur in groups of up to several dozens of objects (Figs. 6a, 8a). The diameters of a total of 108 objects were measured in several images that showed groups of objects (like Fig. 6a–d) with magnifications in the range of 10–20,000×. All well-exposed objects that have the typical globular shape were measured. The measurements produced an average size of 0.8 µm with a standard deviation of 0.17 µm (Fig. 7). The individual globular objects within a group can occur isolated, as dumbbell-shaped pairs, as chains or as compact clusters. Elongate coccoid objects were observed as well, both as isolated individuals, and as connected pairs (Figs. 6b–c, 8b–e).

The surfaces of most of the objects appear smooth or at the most slightly granular. Objects with a distinctly rough surface were observed in the non-coated sample (Fig. 8b, c). Of these, some of the larger ones form dumbbell-like pairs of globules. These are associated with smaller (≤0.5 µm) coccus-shaped objects, with a similar surface roughness and clusters of star-like crystals, probably haematite (Fig. 8a, f). Similar crystals were reported by Trewin and Knoll (1999), in association with fossilised Devonian microbes in calcite veins. Several of the globular objects show a circular structure (Fig. 6b–d), possibly marking a former connection to another object in the chain or cluster. One coccoid object was observed with two parallel bands girdling it (Fig. 6e).

Filaments are occasionally found in association with these objects. These can be mucus-like bundles of strings (Fig. 6f), or about 0.1 µm wide threads with knots (Fig. 8d, e). The threads connect the coccoid or globular objects, as well as irregular flattened mucus-like patches of similar size that resemble collapsed objects (Figs. 6g, 8d).

Because of their small size, we have not been able yet to accurately determine the chemical composition of the objects. EDS-analyses of the vein calcite show, as expected, only strong Ca, O and Au peaks, consistent with the $CaCO_3$ composition of calcite and gold coating. Low-magnification EDS element maps show small non-calcite mineral grains dispersed through the veins, especially in the median zone, as was already observed optically in thin sections. These minerals include quartz and other wall rock inclusions, as well as some sulphides.

Spot measurements inside the objects show almost no difference with the surrounding calcite signal. Apart from Ca, O and C signals, only a weak additional S signal was observed (Fig. 9). A problem is that the S-peaks lie very close to the Au-peak at about 2 keV. However, subtraction of the signal outside an object (pure calcite) from the

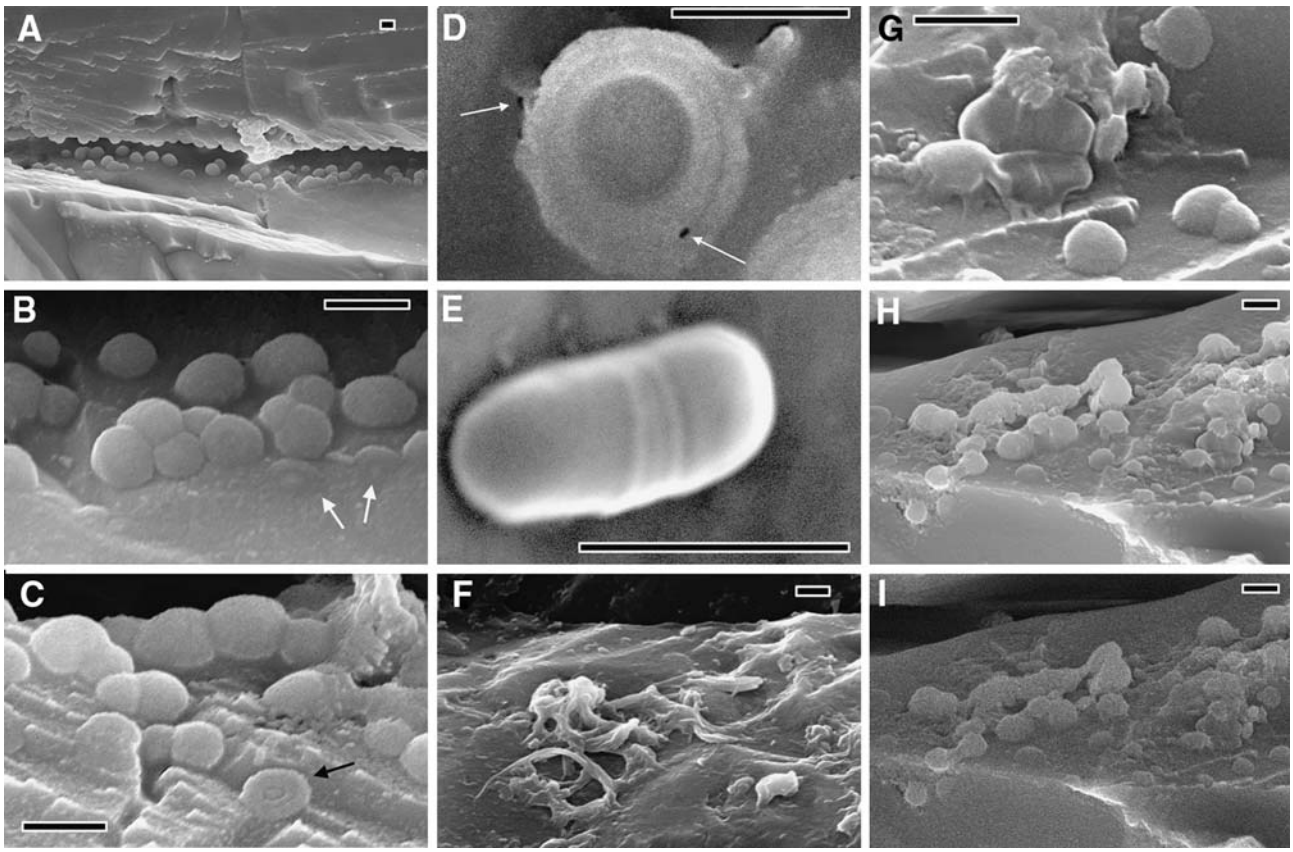

Fig. 6 SEM images of gold-coated samples. **a** Colony of ~1 μm sized globular objects. Etching proceeded deeply along a fibre boundary, revealing the objects and leaving a crystallographically controlled jagged surface. **b**, **c** Clusters and chains of individual globular objects. Some objects are only partly exposed (*white arrows* in **b**), showing that the objects were completely embedded within the calcite before etching. Several globules have a circular marking on their surface (*black arrow* in **c**). **d** Detail of globular object with double circular structure or rim of unknown origin. Small dark specks (*white arrows*) are the result of small holes in the gold coating, showing that gold coating is much thinner than the globular objects. **e** Elongate coccoid object with double girdle bands. **f** Mucus-like filaments. **g** Close-up of individual globular objects and patch that resembles a collapsed globule (detail of **h**). **h** Group of globular objects and irregular mucus-like patches. **a**–**h** are all secondary-electron images. **i** Backscatter image, retrieved at maximum contrast setting on the SEM, and subsequently digitally contrast enhanced to show that the objects and mucus show no significant brightness contrast with the surrounding calcite, and must therefore be of about the same density. All images, except **f** from etched samples. All images taken at a voltage of 15 kV, except for (**e**, **f**) taken at 30 kV. All *scale bars* 1 μm

signal inside an object shows the unequivocal presence of sulphur in the objects (Fig. 9d). Sulphur is also the only element where element mapping showed a noticeable difference between object and calcite matrix (Fig. 10). Sulphur is clearly enriched within the objects. This cannot be an effect of the roughness of the surface, as other elements do not show this effect. However, it should be stressed that the objects are too small to obtain quantitative measurements from the objects only, as the signal always includes the surrounding calcite signature. The composition of the objects is thus constrained to contain one or more of the elements Ca, O, C and S. Hydrogen cannot be measured with the technique, but may be present as well in the objects. The data significantly limit the possibilities for the composition of the objects. Pure calcite (or aragonite) can be excluded, because the objects are resistant to HCl etching. Elemental sulphur is highly unlikely, as it is never found in such veins. Common sulphides, such as pyrite, chalcopyrite or galena contain elements (Fe, Pb, Cu) that, if present, would clearly show up in the EDS measurements, but that show no enrichment whatsoever in element scans. The observed elements could be consistent with the globules consisting of Ca–sulphate, either gypsum ($CaSO_4 \cdot 2H_2O$) or anhydrite ($CaSO_4$). Gypsum can be ruled out, as it falls apart under the electron beam, because of its crystal water. This leaves anhydrite as the only viable mineral that could make up the objects. It should, however, be noted that anhydrite crystals were not observed in the fluid inclusions, nor anywhere else in the veins or their host rock. An alternative possibility is that the objects contain organic material, consisting of C, S, and possibly H and O.

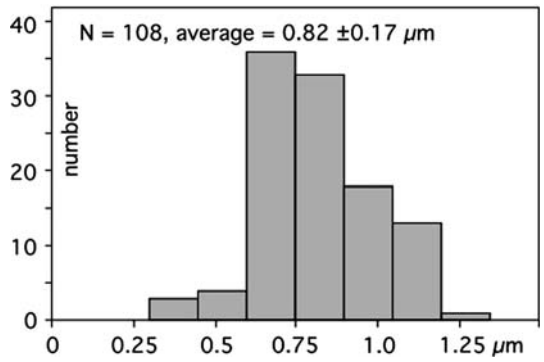

Fig. 7 Histogram of diameters of 108 globular objects from groups in several images such as Fig. 6a. All well-exposed objects that have the typical globular shape were measured. For each object the average diameter was measured on the SEM images, or estimated if the object was still partly embedded in calcite. The group of distinctly small and elongate objects shown in Fig. 8a (size range 0.3–0.6 μm) is not included

The objects, apart from the bright granular ones with ?haematite crystals, appear relatively dark in FE-SEM images of the uncoated sample (Fig. 8d). In backscatter mode, however, the clusters of objects show no significant brightness contrast with the surrounding calcite matrix (Fig. 6h, i). Therefore, the density contrast between objects and calcite matrix is at the most minor.

Interpretation

The central question to be asked in this section is "what are the ~1 μm sized objects that were observed with the SEM?". To address this question, we will first have to answer the question on *when* the objects were introduced into the veins. The possible timing of the object formation can be (1) during sample preparation and handling (i.e. they are artefacts or contaminations), or (2) after vein formation, but before sample collection, (3) before vein formation or (4) synchronous with vein formation. Having established the age of the objects, which we will argue is most likely the same as the vein formation, we will briefly revisit the age determination of the veins.

Next, we will discuss the nature of the objects: what are they? Because of their morphological resemblance to microbes, one hypothesis (A) is that they are fossil microbes. Another hypothesis (B) is that they are the product of some purely abiotic process. The third hypothesis (C) is that they are artefacts resulting from sample treatment or analysis. Hypothesis (1) for timing directly relates to hypothesis (C) for the nature of the objects, and can be summed up as the hypothesis that the objects are recent artefacts or contaminations.

Claims of the discovery of mineralised extraterrestrial life forms on a Martian meteorite (McKay et al. 1996) and

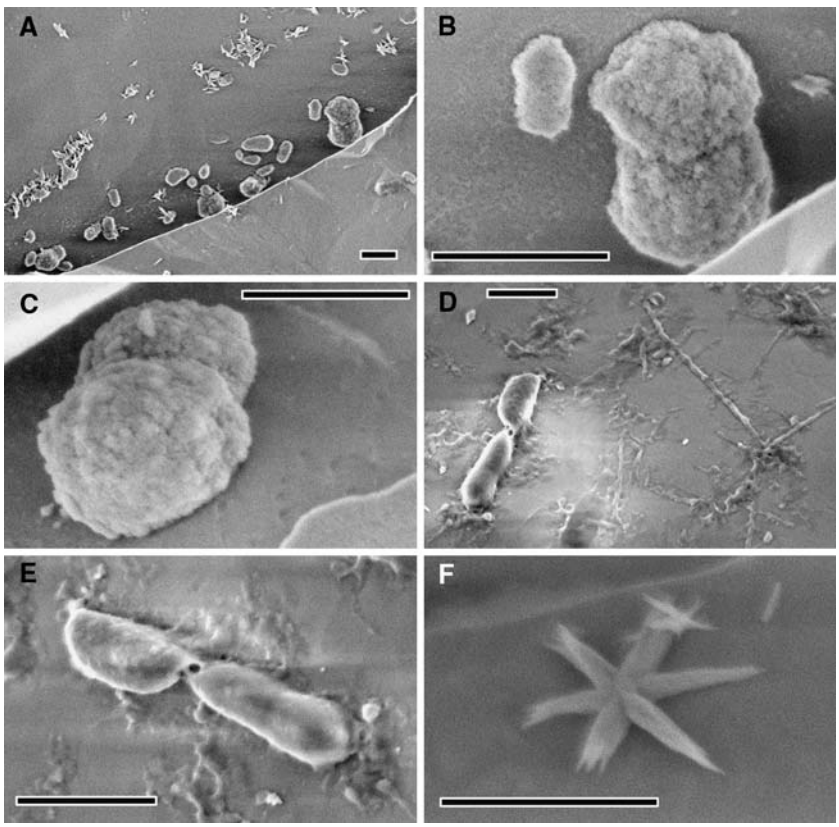

Fig. 8 Field-emission SEM images of an *uncoated* fibrous vein. **a** Cluster of rough-surfaced elongate objects, most ~0.5 μm and the larger ones about ~1 μm wide. The cluster is associated with a row of small needle- and star-like crystals, probably Fe-(hydr-)oxide. **b**, **c** Close-up of objects from the group shown in **a**, showing different double spheres, with radial constriction, with rough, granular surface structure. **d** Distinctly coccoid objects, resembling a chain of cells. The objects are connected to thin filaments with small knots. Some of the filaments disappear into the calcite, showing that they are encased within the calcite and were exposed by etching. **e** Close-up of the two coccoid objects shown on the left in image (**d**). **f** Close-up of a 6-pointed star-shaped crystal, associated with the objects shown in **a**. All images taken at 2 kV; all *scale bars* 1 μm

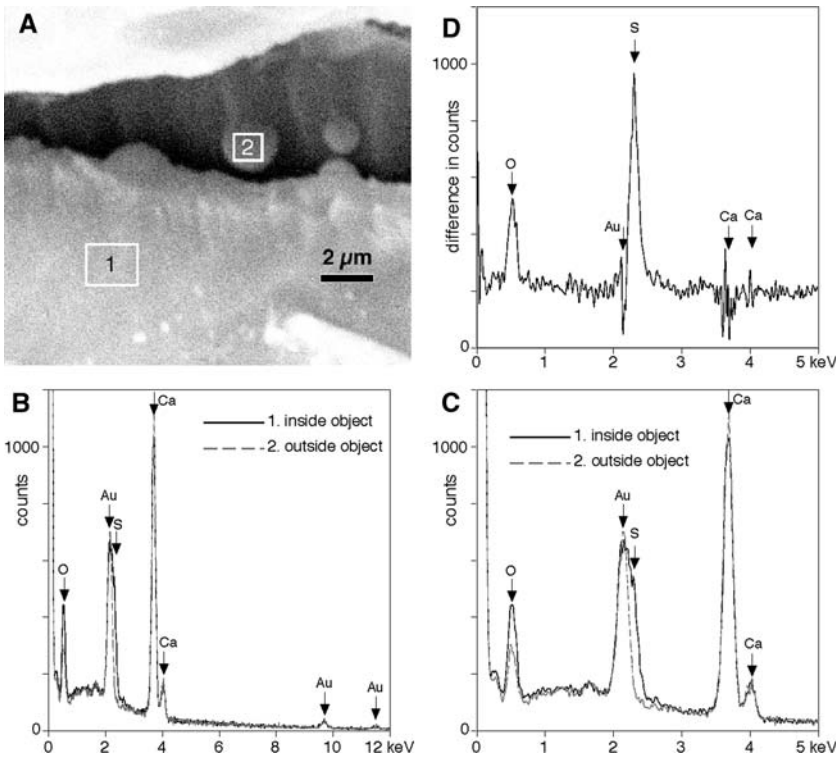

Fig. 9 **a** Locations of EDS measurements inside an object (box 2) and in the host calcite (box 1). EDS spectra from both locations showing the dominance of O, Ca and Au peaks. **c** 0–5 keV detail of the two spectra. The only significant difference between the two spectra is the wide right shoulder of the Au-Mα peak, signifying the presence of S (Kα-peak) inside the object. **d** Difference between the two spectra, proving the presence of S in detectable amounts inside the object

the debate on the oldest fossil microbes (Schopf 1993; Brasier et al. 2002) have highlighted the need for very careful scrutiny of any suspected microfossils (Schopf 1992, 1999; Westall 1999; Altermann 2001; Brasier et al. 2002; García-Ruiz et al. 2002, 2003; Cady et al. 2003). Altermann (2001) summed up the requirements for recognising an alleged (Archaean) microfossil as such: "To be regarded as bona fide, microfossils must be demonstrably structurally preserved, unquestionably biogenic, and both indigenous to and proveably syngenetic with the primary deposition of the [sedimentary] rock embedding them." Furthermore "It is crucial to demonstrate that the declared [Archean] microfossils are indeed not microbial, mineralogical or artificial contaminants and artefacts introduced to the rock after its formation." (square brackets added, because the objects here are neither Archaean, nor are they embedded in sedimentary rocks). In the discussion below on the interpretation of the objects, we will try to address all the issues raised by Altermann (2001) and other authors.

Age of the objects

Hypothesis 1: The objects are recent artefacts or contaminations

It is known that sample preparation, especially for the SEM, can introduce artefacts that may be mistaken for microbes (Kirkland et al. 1999). Several authors therefore prefer optical analysis of thin sections (Altermann 2001; Cady et al. 2003). It should, however, be noted that the objects here are about 1 μm in size, which is far too small for any meaningful optical analysis in petrographic thin section. Can the objects be the result of etching? Kirkland et al (1999) showed that etching can produce "biomorph" structures in calcite. These are however distinctly smaller than the ~1 μm size object observed here. In our case, the etching of the calcite produces a distinct type of roughness, related to the crystallography of the calcite. The objects are positive, i.e. they stand out from the etched calcite surface, and were therefore resistant to etching, and have a slightly different chemical composition than the calcite (presence of sulphur, etching resistant). The brief etching process with HCl cannot create positive, sulphur-bearing objects that are often partly buried in the surrounding calcite. Etching artefacts can therefore be excluded. Conductive gold or carbon coating is another well-known possible source for artefacts (Folk and Lynch 1997). Because the objects were also observed in an uncoated sample (Fig. 7), coating artefacts can be excluded. To avoid producing any artefacts in samples that were gold-coated, sputtering time was kept to the minimum time (30 s) needed to achieve sufficient conductivity. This produced a very thin gold layer, much thinner than the globular objects. This can be seen in Fig. 6d, where small holes in the gold coating (white arrows) show that the objects themselves are not composed of gold blobs.

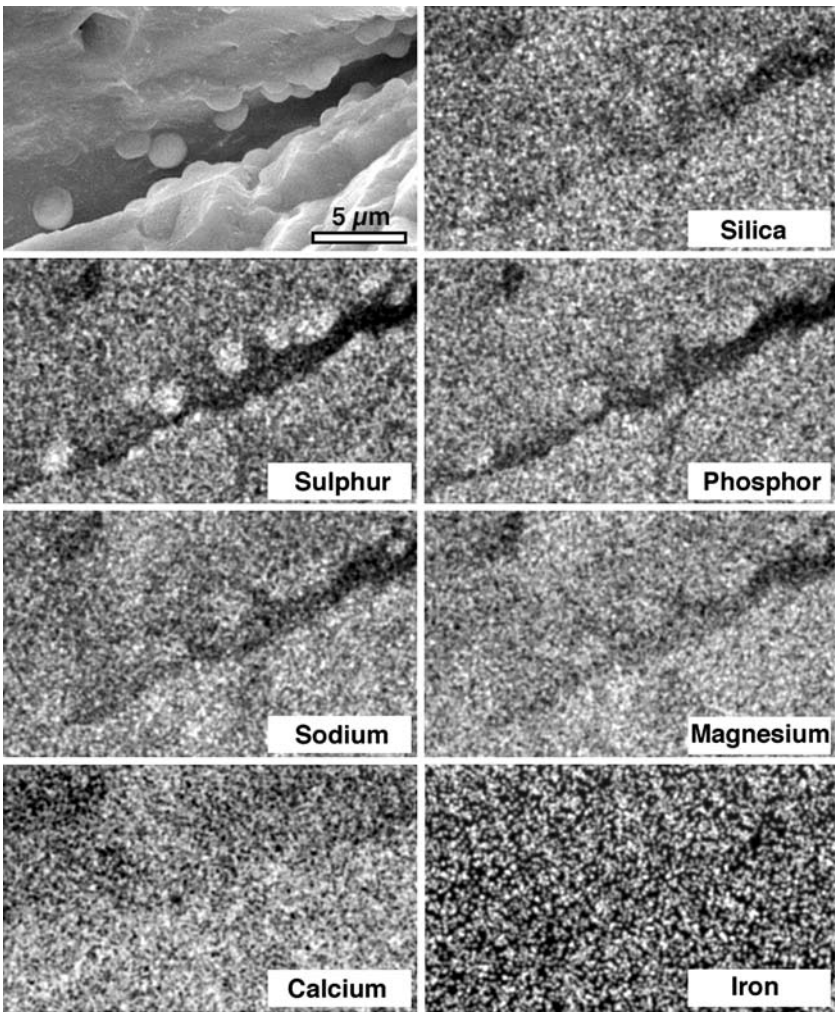

Fig. 10 Secondary electron image of a number of globular objects revealed by etching, together with EDS element maps for selected elements. Sulphur is the only element that shows any distinct difference in composition between objects and surrounding calcite. The brightness (enrichment in S) is not an effect of the surface morphology, since the rough edges in the lower right of the image do not produce any apparent variations in S-concentration

Contamination by dust or microbes after polishing and etching the sample can be excluded on the basis that many objects are partly embedded inside the vein calcite. Contamination after gold coating also shows up by bright charging effects. In conclusion, the possibility that the objects are recent (laboratory) artefacts or contaminations can be rejected.

Hypothesis 2: The objects formed after vein formation, but before sample collection

The fact that many objects are still partly embedded within the vein calcite is of crucial importance to address the hypothesis that the objects were introduced into the veins after vein formation, but still by natural processes before sampling. The objects must thus be at least as old as the youngest calcite that contains them. This leads to the question "was there any calcite precipitation or recrystallisation in the fibres after the primary vein formation?". Different stages of calcite precipitation is normally visible by (a) different cathodoluminescence (CL) of the different calcite generations and (b) microstructure. No variation in CL was found that could indicate any reprecipitation or recrystallisation of calcite (Elburg et al. 2002). The smooth fibre boundaries (a high grain boundary surface energy configuration) are further evidence against any recrystallisation or reprecipitation. The fibre boundaries are tight (Fig. 5b), meaning that any space between them is far less then 1 μm, which makes it impossible for an object of ~1 μm to have entered the veins after fibre formation. All these observations indicate that the fibrous morphology is primary and that the objects must therefore be at least as old as the primary fibre calcite.

Hypothesis 3: The objects formed before the veins

Fibrous veins, such as the ones at Oppaminda Creek, invariably contain wall rock inclusions (Hilgers and Urai 2005). These inclusions, of variable size, are optically visible and with the SEM. It can be envisaged that existing objects were included in the vein from the wall rock by the same mechanism. None of the observed objects is associated with recognisable wall rock inclusions. Instead, objects are always surrounded by pure calcite. It is unlikely, but not

impossible, that the micron-sized objects (mineral grains? fossil microbes?) were plucked from the wall rock without any wall rock mineral grains. Alternatively, small clumps of material could have been washed into the vein by percolating fluids. This is theoretically possible since Elburg et al. (2002) showed that at least part of the calcite must have been derived from fluids introduced from outside of the system. However, it is extremely unlikely that clusters of objects, together with threads or filaments (Fig. 8d), would have been carried into the growing veins without any associated other wall rock particles.

Hypothesis 4: The objects are of the same age as the veins

It was already argued above that the objects must be of at least the same age as the fibrous calcite and that the fibrous calcite is of the same age as the veins. This implies that the possibility that the objects are of the same age as the veins cannot be excluded, and appears as the only viable option.

Re-evaluation of the age of the fibrous veins

The age of the objects is constrained as equal or greater than the age of the fibrous calcite in the veins. The age of the veins is so far only constrained at about 585 Ma by the Sr-isotopic data of Elburg et al. (2002) and the indications of a basin wide event at that time (Foden et al. 2001). Other direct age determinations on the veins are lacking. However, circumstantial evidence also points to a very late Neoproterozoic age, predating the ~500 Ma Delamerian Orogeny. Some veins are syntaxial, not fibrous and carry calcite, dolomite and quartz. Fibrous veins may have parts of this vein type. This second vein type is found to be younger than the fibrous veins by overgrowth and cross-cutting relationships. Whereas fluid inclusions indicate a formation temperature of between 60 and 100°C for the fibrous veins, the younger veins formed at higher temperatures. This means that the high-temperature event succeeded the low-temperature event. There are only two known thermal events in the region: the ~500 Ma Delamerian Orogeny (Mildren and Sandiford 1995; McLaren et al. 2002) and a ~440 Ma thermal event (Elburg et al. 2003). Although McLaren et al. (2002) argued for a single event that spanned the whole period (500–440 Ma), Elburg et al. (2003) favour two distinct thermal pulses. It is most likely that the high-temperature phase of carbonate and quartz precipitation occurred during one of the two early Palaeozoic thermal events, in which case the low-temperature fibrous veins must have formed at the latest between 500 and 440 Ma in the Elburg et al. (2003) model, or before 500 Ma in the McLaren et al. (2002) model. The fibrous vein formation at a temperature of 60–80°C indicates a burial depth of at least a few kilometres at a normal thermal gradient. This is in line with the estimated 585 Ma burial depth of 3-6 km. In conclusion, the possible age range can be bracketed by the 643 Ma age of sedimentation of the host rock and the 500–440 thermal overprint. Without any new data, the best estimate of the age remains about 585 Ma.

Bons and Montenari (2005) showed that the orientations of the veins and their fibres show a consistent evolution over time. The orientation of veins is related to the nucleation of veins in cracks, which is in turn related to the minimum stress orientation, or extension direction. The orientation and curvature of fibres is related to the direction of divergence of the vein walls, which is also related to the extension direction. A progressive over 90° rotation of vein nucleation orientation and fibre growth direction indicates a significant rotation of the stress field, which suggests a prolonged history of fibrous calcite growth, possibly tens of millions of years in line with the only direct measurement of fibre growth rates (Mueller et al. 2000).

Interpretation of the objects

We will now address the origin of the objects, discussing the alternative options that the objects are (A) fossil microbes or (B) the result of some abiotic chemical-physical process. We will discuss four aspects that need to be addressed and for which data are available: conditions of formation, morphology, size range and chemistry of the objects.

Conditions of formation of the fibrous veins

Fluid inclusions indicate a range of 60–80°, not exceeding 100°, for the temperature of vein formation. This is in line with the temperature conditions estimated from the stratigraphic burial depth at the ~585 Ma time of vein formation. These temperatures are well within the range amenable to life. Life is known to survive temperatures of over 100°C in hot springs, fumaroles, and black smokers (Stetter et al. 1983, 1993; Huber et al. 1989, 1990; Jones et al. 1996; Blöchl et al. 1997). Present-day life is found to a depth of several kilometres in the subsurface (Stetter et al. 1993; Liu et al. 1997; Kerr 2002; Lin et al. 2006). The conditions, at least in terms of depth and temperature, were therefore amenable to life at the time of fibrous vein formation.

Morphology of the object

Morphology is a controversial indicator for the authenticity of fossil microbes (e.g. García-Ruiz 1998; Westall 1999;

Altermann 2001; Brasier et al. 2002; García-Ruiz et al. 2002; Cady et al. 2003), since purely abiotic processes may produce remarkably life-like structures. For example, García-Ruiz et al. (2002; 2003) experimentally produced filaments and related biomorph structures in silica, which bear a strong resemblance to the putative fossil cyanobacteria in the Apex Chert (Schopf 1993). Unfortunately, most work on fossilisation of microbes or the formation of similar-looking pseudo-fossils has concentrated on siliceous host rocks, which have a better preservation potential for microbes than carbonates (Konhauser et al. 2003). Several authors (e.g. Cady et al. 2003) stress the need to also prove that the alleged microfossils are composed of organic material of biosynthetic origin. In their view, microbes that are completely replaced by minerals cannot be distinguished from abiotic pseudo-fossils. However, in the same way that one cannot prove that a structure is a fossil microbe because it looks like a fossil microbe, one cannot prove that a structure is abiotic because of its appearance. If structures are biotic in appearance, a biologic origin should be considered as a possibility.

The objects have a simple basic coccoid or globular morphology (Figs. 6, 8). This morphology is similar to that of encapsulated microbes in calcite precipitation experiments (Bosak et al. 2004) and microfossils embedded in pyrite (Schieber 2002). Some objects show radial constrictions (Fig. 8e) or girdles (Fig. 6e) similar to wall bands that form during cell division (see Fig. 3.39, p. 76 in Madigan et al. 2000). In Fig. 8d, e the objects are seen to be associated with thin filaments, reminiscent of the modern hyperthermophile archaea *Pyrodictium* (Stetter et al. 1983; König et al. 1988). These filaments form a network, spanning three coccoid objects and dark patches of similar size as the coccoid objects. These patches resemble collapsed objects (Fig. 6g). Although spheres, rods, strings and filaments can individually all form by abiotic processes (García-Ruiz et al. 2002, 2003), their *association*, as well as the general absence of associated clearly abiotic structures, is what is to be expected for fossilised microbes of the $\sim$1 μm size range. Although the objects are morphologically not "unquestionably biogenic" in the words of Altermann (2001), the alternative is to invoke a hitherto unknown abiotic process that produces such structures (containing S) in a Ca–carbonate matrix.

Size range of the objects

A particular aspect of life forms is that they have a species-specific size range with a positive skewness in a size distribution (Strother 1996). The size range of the objects in our samples is between about 0.5–2 μm, which is compatible with that of bacteria or archaea (Pirie 1973; Koch 1996; Nealson 1997). For example, the hyperthermophile archaea reported by Huber et al. (1990) range in size from 0.3 to 5 μm. A histogram of the diameters of 108 globular objects shows a narrow spread around 0.8 μm, and a positive skewness of the histogram (Fig. 7).

Abiotic processes may also produce structures (e.g. mineral grains) of a particular size, which is determined by the balance of different controlling factors, such as diffusion rates, nucleation, surface energy, etc. A narrow size range can indeed occur if the balance remains constant, which is the case if the conditions determining that balance remain constant. If the objects are abiotic, a constant narrow size range of the objects in samples from different veins would thus imply the unlikely case that the size-controlling factors remained roughly the same between different veins and over the possibly prolonged time of vein formation.

Chemical composition of the objects

The chemical composition of the objects is constrained to the elements S ± Ca ± C ± O by EDS analysis. Of these elements, only sulphur is certainly part of the objects, as the other element signals may derive from the surrounding calcite. Since hydrogen could not be measured, a possible ±H should be added to the element list.

If the objects are inorganic minerals, the only reasonable mineral candidate is anhydrite ($CaSO_4$). Although it may theoretically be envisaged that anhydrite forms biotic-looking structures in carbonate, similar to, for example, the witherite ($BaCO_3$) structures in silica reported on by García-Ruiz et al. (2003), we are not aware of any reports of that kind for anhydrite. We have not observed any larger anhydrite crystals inside the vein calcite, nor in the fluid inclusions or any outcrop in the area. An anhydrite composition of the objects should therefore be regarded as unlikely.

The alternative is that the objects are composed of sulphur-bearing organic matter (S + C ± H ± O). We can envisage two possible origins for the objects if they are composed of organic material. The first is that the objects are fossil microbes. This would be a possibility in line with the morphological data discussed above. A second option is that the objects were formed by some abiotic process. In that case, one possibility is that the objects were carried along by a percolating fluid during vein formation. Although stratigraphic units below the Tindelpina Shale Member are not noted for their high content of organic matter, the shales themselves are rich in organic matter (McKirdy et al. 1975). Such an advective origin of the organic matter may thus be possible, but unlikely as a hitherto unknown process must be invoked to explain their

particular morphology, size range and occurrence in clusters and colonies. The simplest interpretation of the chemistry of the objects appears to be organic and biotic, but an alternative abiotic origin could theoretically be envisaged, even though this calls for more unknown processes to have taken place.

Summary

The above discussion of the data, summarised in Table 1, show that both hypotheses on the nature of the objects (they are fossil microbes versus they are not fossil microbes) are possible, because neither can be proven wrong. A qualitative assessment of the likelihood that one of the two hypotheses is true would favour the interpretation of the objects as fossil microbes, because this interpretation does not rely on invoking any unknown processes.

Discussion

The observations presented in this paper suggest the possibility that the veins were inhabited by microbes at the time of their formation, around 3–6 km below the seafloor and about 585 million year ago. The possible existence of such microbes is by no means controversial, as life is known to inhabit the deep subsurface (Pedersen 1993; Stetter et al. 1993; Liu et al. 1997; Kerr 2002; Stetter 2002), and is likely to have done so for a long time. The novelty of our findings is that fossilised microbes of such an age and original depth have so far not been reported on.

One problem in the search for ancient deep-subsurface microbial life lies in recognising the fossilised remains of such microbes (assuming they can actually be preserved as fossils), and targeting the right rocks to search for them. Mineral veins, as were investigated here, are particularly prospective, since they represent former sites that provided space for organisms to live in, as well as elevated fluid percolation to bring the necessary nutrients (e.g. Hofmann and Farmer 2000; Budai et al. 2002). Mineral precipitates inside veins also form a stable matrix to encapsulate microbes (Cady et al. 2003) and enable their preservation over geologic time. If the particular antitaxial fibrous habit of the calcite veins is in some way related to microbial activity, this would open up the opportunity to specifically target this type of veins as particularly prospective hosts for fossil microbes.

Table 1 Summary of the arguments used in the discussion of the timing (a) and the biotic versus abiotic origin (b) of the objects

(a) Statement on timing of object formation	Outcome	
1. The objects are recent artefacts or contaminations	Rejected	
1a. The objects are artefacts from etching	Rejected	
1b. The objects are artefacts from gold coating	Rejected	
1c. Contamination by dust or microbes after sampling	Rejected	
2. The objects formed after vein formation, but before sample collection	Rejected	
2a. Calcite precipitation or recrystallisation occurred after primary fibrous calcite growth	Rejected	
3. The objects are older than the veins	Possible	
3a. The objects are wall rock inclusions	Unlikely	
3b. The objects were introduced by percolating fluids	Unlikely	
4. The objects are of the same age as the veins	Acceptable	
b) Statement on origin of objects	Biotic	Abiotic
The conditions of formation were amenable to biotic/abiotic processes	Yes	Yes
The morphology is compatible with a biotic/abiotic origin	Yes	Possible
The size range is compatible with a biotic/abiotic origin	Yes	Possible
The chemistry is compatible with a biotic/abiotic origin	Yes	Possible
The objects are composed of inorganic anhydrite	–	Unlikely
The objects are composed of organic material formed inside the veins by biotic/abiotic processes	Yes	Unlikely
The objects are composed of organic material introduced to the veins	–	Unlikely
The objects have a biotic/abiotic origin	Acceptable	Possible

Acceptable the statement cannot be rejected, *rejected* the statement can be rejected, *yes* statement can be true by invoking a known process, *possible* statement can be true, but by invoking an unknown process, *unlikely* statement can be true, but with a small likelihood, because the (unknown) process is improbable, or unlikely to be compatible with other observations

As was referred to above, the determination of fossil microbes is extremely difficult and often controversial. This is already the case for fossil remains of Archaean cyanobacteria (see Westall 1999; Altermann 2001; Cady et al. 2003 for critical assessments of the criteria to recognise microfossils) that are in the 10-µm-size range. The difficulties are compounded when the suspected fossil microbes are only about one micron in size, as is the case here. However, this should not keep us from considering the possibility that such small fossil microbes could be preserved in ancient rocks (Schieber 2002; Bosak et al. 2004), and may be revealed by a combination of several analytical techniques. In our view, the globular objects presented in this paper are too biomorphic to exclude the possibility of a biologic origin. Although the final verdict on their true nature must remain open, we hope our results will encourage investigations of possible remains of ancient deep life in (antitaxial fibrous) veins that have hitherto never been considered as a host to microbial life.

Conclusions

A detailed micron-scale electron-microscopic investigation of antitaxial fibrous calcite veins from Oppaminda Creek (South Australia) revealed micron-sized globular and coccoid objects. Their morphology, size and available chemical data are supportive of the interpretation that these objects are fossil microbes. These microbes would have inhabited the veins at the time of their formation at a depth of 3–6 km, and an age of about 585 Ma. Fluid inclusions and age-stratigraphic arguments constrain the formation temperature as less than 100°C, most likely about 60–80°C.

Our discovery of suspected fossil microbes of significant age and original depth of formation opens up new prospective host rocks to be considered for the search of remains of extraterrestrial life or ancient microbial life forms on Earth. The fact that fibrous veins are fibrous means that the vein-forming minerals are primary. Fibrous veins also normally grow at relatively low temperatures. Fibrous veins should therefore be regarded particularly prospective hosts to find preserved remains of microbes.

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